FUNCTIONAL MICROSTIMULATION OF THE LUMBOSACRAL SPINAL CORD

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ABSTRACT

The main aim of this contract is to test the idea that intraspinal microstimulation (ISMS) can be used selectively to excite neurons that activate the bladder detrusor muscle while simultaneously stimulating interneurons which inhibit motoneurons of the external urethral sphincter (EUS). If this reciprocal action works well enough to produce bladder voiding after spinal-cord-injury (SCI), it could form the basis of a neuroprosthesis that would restore bladder control without the need for transection of sensory nerve roots of the spinal cord (dorsal rhizotomies).

In this second quarter of operation the following was achieved:

1) The following hardware items were developed and tested in two acute experiments and one chronic implant:
   - A custom flow measurement system (force transducer under a weighing pan).
   - A modified pediatric Foley catheter for intra-vesicular pressure monitoring.
   - A sacral back-pack to house the percutaneous portions of implanted leads, catheters and connectors.

2) In two acute experiments:
   - We tested the hypothesis that intra-urethral EMG is proportional to urethral pressure and could therefore be used in lieu of pressure transducer measurements. The hypothesis was not supported. Pairs of EMG signals recorded simultaneously along the urethra revealed large differences in local EMG activation.
   - The effect of intra-urethral stimulation on urethral and bladder pressures was measured using the indwelling Cooner wires described in the previous quarterly report. Depending on bladder pressure and stimulus amplitude, urethral pressure could either increase or decrease without significant changes in bladder pressure. Intra-urethral stimulation might therefore be useful in relaxing the urethra to allow voiding of the neurogenic bladder, or alternatively, in contracting the urethra to maintain continence.
   - Increases in bladder pressure were elicited with ISMS from numerous sites within the sacral spinal cord. Increases or decreases in urethral EMG were also elicited from the same or other sites. Decreases in urethral EMG were not necessarily accompanied by decreases in urethral pressure. In general ISMS sites that inhibited the urethra were far harder to find than those activating the bladder, as we reported previously (Prochazka et al. 2002). Trains of interleaved ISMS pulses delivered at urethra-inhibitory and bladder-excitatory sites unfortunately always resulted in urethral activation.

3) The first chronic implant of a multichannel ISMS elecrode array was performed. The array was tested some days after implantation with the animal either awake or under light anesthesia. The following observations were made:
   - Increases in bladder pressure of up to 40 mm Hg were elicited by ISMS in the awake animal without any signs of discomfort.
   - However the bladder contractions were accompanied by intra-urethral EMG responses indicating urethral co-contraction. Consistent with this, no voiding occurred.

- ISMS through the microwires implanted more rostrally to target the dorsal commissural region that inhibits urethral activity elicited aversive reactions in the awake animal. As soon as these occurred ISMS was discontinued. Consequently we were unable to test combinations of urethral-inhibitory and bladder-excitatory ISMS under these conditions.

PROGRESS IN THIS QUARTER

METHODS

Anesthesia and Monitoring of Vital Signs

Three male adult cats were used in the experiments. In the two acute experiments the cats were anesthetized with 2-3% isoflurane in carbogen: (95% O_2, 5% CO_2), 1.5 L/min. A tracheostomy was performed and anesthesia was maintained through a tracheal tube. Blood pressure was monitored with a pediatric sphygmomanometer cuff (4 cm) wrapped around the left upper arm and attached to a Cardell 9301V pressure monitor. A cannula was inserted in the left jugular vein to allow for the administration of fluids. The cats' body temperature was maintained using heating pads and lamps. One cat was decerebrated after measurements had been made under anesthesia. In this case both carotid arteries were ligated with 2-0 silk suture. After decerebration anesthesia was discontinued. Dextran (10 ml/kg, 10% dextran in 5% dextrose lactate ringer's solution) was administered through the jugular vein when the mean blood pressure dropped below 80 mmHg.

In the third (chronic implant) cat, ISMS microwires were implanted in the sacral spinal cord as detailed below. The implant procedure was also performed under isoflurane anesthesia as described above. A pediatric endotracheal tube was inserted and the anesthetic mixture was delivered in closed-loop mode. An intravenous catheter was inserted in the cephalic vein and a saline drip was delivered throughout the procedure. The surgery was performed in a fully-equipped operating room with sterile equipment and procedures.

Back-pack

In our contract application we proposed a detachable head-piece designed to house the connectors from the microwire array, intra-urethral EMG wires and intravesicular catheters. However recently in another project involving chronic dorsal root implants we have used an external back-pack fixed percutaneously to the sacrum. Because this is physically much closer to both the bladder and sacral spinal cord we thought it worth trying out for the current application. The two-part back-pack is shown in Figs. 1 and Fig. 2. It consisted of a subcutaneous stainless steel saddle (Fig. 1A) that was first screwed to the iliac crests on each side of the

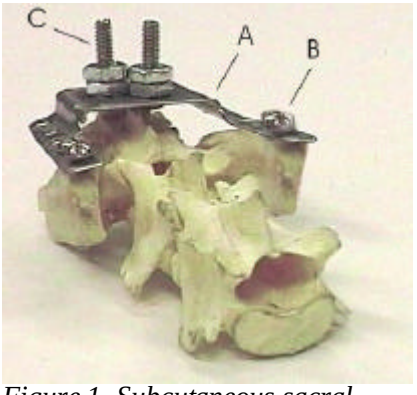

Figure 1 Subcutaneous sacral saddle (A) screwed to ilac crests (B)with percutaneous bolts (C) to fixate external enclosure

sacrum (Fig. 1B). After the ISMS microwires had been implanted in the spinal cord and the paravertebral muscles and lumbo-dorsal fascia had been sutured closed with resorbable catgut, the skin wound was sutured shut over the saddle, with two fixation bolts (Fig. 1C) attached to the saddle emerging through holes in the skin. The terminal connectors of the wires and catheter that were also led through the skin were placed inside a flat box (Fig. 2A) through holes in its base. The box was then attached to the saddle bolts with nuts and closed with a lid (Fig. 2B).

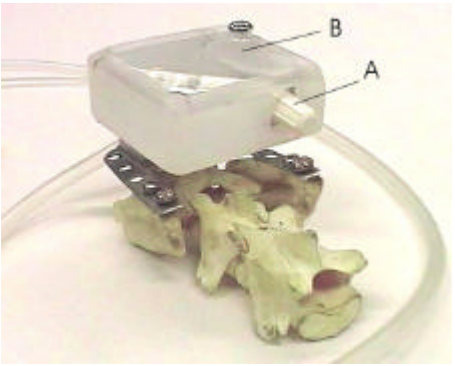

Figure 2 External enclosure attached to saddle, showing catheter connector A) and transparent lid (B).

Implantation of Urinary Tract Monitoring Devices

This quarter we used Foley catheters (Fig. 3) instead of the custom-made catheters described in our first report. Foley catheters have inflatable retaining pouches. This allows them to be inserted through a puncture hole of similar diameter to the catheter itself (2mm), rather than having to allow passage of the internal retaining button on the previous catheter. The bladder wall tends to self-seal around the Foley catheter so purse-string sutures are eliminated, reducing trauma to the bladder wall. The Rusch 2 mm Foley catheter we selected is 29 cm long, which is not quite long enough to reach from inside the bladder to the back-pack after allowing some slack for strain relief. We therefore lengthened it by an extra few centimeters with 4mm diameter silastic tube bonded with RTV elastomer (see Fig. 3B). The side-arm used to inflate the retaining pouch has an internal valve which could be implanted subcutaneously (Fig. 3C). The extension tube was terminated with a Luer hub and cap for stowage in the back-pack enclosure.

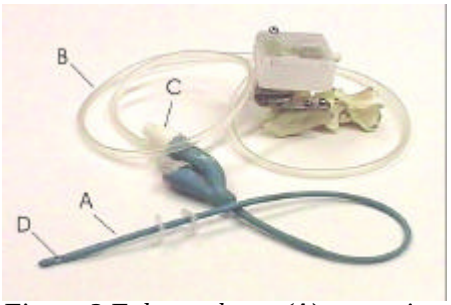

Figure 3 Foley catheter (A), extension tube (B), side-arm and valve (C), side-port (D).

The back, abdomen, perineal region and thighs were shaved. The bladder was exposed through a midline abdominal incision. The tip of the Foley catheter was introduced into the bladder through a stab incision made with a 16G hypodermic needle. We found that stiffening the tip of the catheter (Fig. 3D) by temporarily inserting a thin dental probe through its side-port greatly facilitated its entry into the bladder. The probe was withdrawn without pulling the catheter out and the retaining pouch was inflated with 3 ml sterile saline via the valve (Fig. 3C). The free end of the cannula was tunneled subcutaneously to emerge at the skin incision overlying the sacrum and under the backpack.

Intraurethral EMG electrodes and pressure measurements

In the two acute experiments, the aim was to record intra-urethral pressure and EMG

simultaneously with the use of indwelling catheters. In the first experiment three Cooner AS631 wires were threaded inside a Kendall Argyle 5 Fr. (1.7mm) feeding tube. The ~4mm de-insulated ends of the wires emerged through three side-ports close to the tip as shown in Fig. 4B, and the free ends emerged through a small hole close to the Luer port where they terminated in 1 mm connectors (Fig. 4A). This hole was sealed with RTV silastic. The tip of the catheter was lubricated, inserted into the penis and pushed in 85 mm (arrow in Fig. 4A) so that the tip was located within the bladder neck. The Luer port was connected to a Neurolog NL108D4/10 dome and NL108T4 isolated pressure transducer. The 1 mm EMG sockets were connected to cables leading to a Neurolog NL820+824 4-channel EMG amplifier system. Three EMG signals were obtained by pairing the wires up as follows: 1+2, 2+3. EMG and pressure measurements were made at a series of positions within the urethra, the catheter being withdrawn in 10 mm steps.

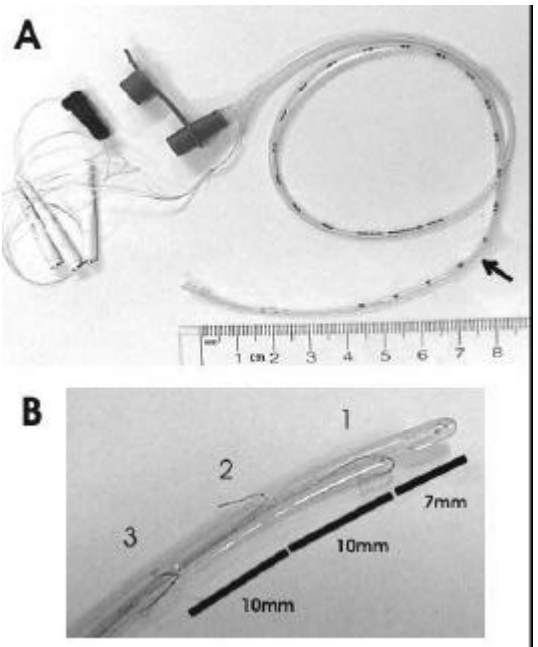

Figure 4 A) Feeding tube catheter with internal EMG wires showing Luer hub for pressure measurements and EMG sockets. B) Detail of end of catheter showing three EMG wires emerging from side-ports.

In the second (acute) experiment one of a pair of Cooner 631 wires was threaded into a Kendall 3.5Fr Tom Cat catheter, the bared end emerging from one of the side ports close to the tip (Fig. 5). The second wire was threaded into one of the ports and its bared tip emerged from the other, so that for most of its length this wire lay alongside the catheter. The pressure and EMG connectors were similar to those in Fig. 4.

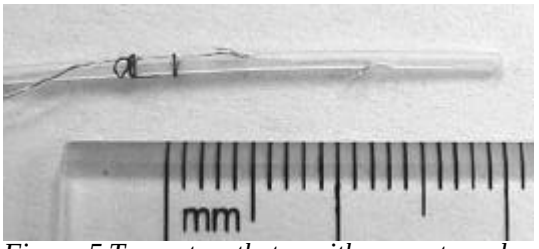

Figure 5 Tomcat catheter with one external and one internal EMG wire.

In the third cat, EMG wires were implanted chronically in the urethra. A length of 1.7mm diameter feeding tube carrying a pair of Cooner 631 wires similar to those in Figs. 4 and 5 was inserted through a puncture hole in the urethra close to the bladder neck and fed through the entire length of the urethra. The tube was pulled out of the penis and the wires were freed from it. The wires were then pulled back into the urethra from the bladder end, leaving their 3-4 mm bared ends at two locations within the urethra, spaced about 10 mm apart. Cyanoacrylate glue was used to seal the puncture hole close to the bladder neck where the wires emerged from the urethra. The wires were tunneled under the skin to emerge through the back wound, where they terminated in a miniature DIL connector that was stowed in the back-pack. During recording sessions this connector connected to cables leading to the Neurolog EMG amplifier.

EMG signals were amplified (gain 1000, bandpass filtered (30 – 2,000 Hz) and digitized at a rate of 4,000 samples per second using a CED Power 1401 (Cambridge, UK) hardware and Signal 2.1 software. The pressure signal was low-pass filtered at 30 Hz and sampled at the same rate. The data were stored on the computer's hard drive for later analysis.

Urethral Flow-meter and Implantation Procedure

A custom-made weighing system based on standard clinical volumetric devices was manufactured and tested It comprised a cantilever strain-gauge force-sensing element from which a light Styrofoam pan was suspended (Fig. 6). The weight of urine in the pan was sensed by the force transducer. The force signal could be differentiated to yield flow. The device has a much better signal-to-noise ration than the system described in our previous report.

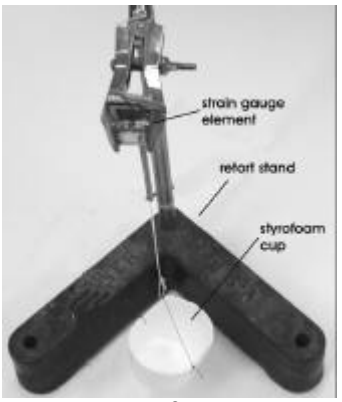

Figure 6 New flow meter.

Laminectomy and Decerebration

In the acute experiments a skin incision was made from the L4 to L7 vertebral spinous processes and a laminectomy was performed to expose spinal cord segments L5 – S1. The cat was placed in a stereotaxic frame consisting of ear bars and a mouth-piece for holding the head, and hip pins and a spinous process clamp (on the L4 vertebra) for maintaining the spinal cord in place. Previous work by our group demonstrated a change in micturition behavior due to anesthesia (Rudy et al. 1991). In one of the two experiments we therefore decerebrated the cat and discontinued anesthesia.

Decerebration was performed using the following procedure:

> The bone of the cranium covering the parietal and occipital lobes on each side of the head (around the tentorial region) was removed using trephine and rongeurs. The dura mater was then opened and a curved spatula was used to remove the cortices and then to transect the brainstem from the rostral edge of the colliculi on the dorsal surface to a postmammillary site on the ventral surface (Fedirchuk and Shefchyk 1991) and a coagulation inducer, Surgicel (Ethicon) was placed in the cranial cavity rostral to the tentorium. Dextran was administered as required to maintain blood pressure above 80 mmHg.

Upon completion of decerebration, the anesthesia was discontinued and the cat was artificially ventilated. Measurement of reflexive urinary tract function and ISMS commenced once decerebrate rigidity developed (~15 min).

ISMS: Locating targets in the Sacral Spinal Cord

In the two ISMS experiments and the chronic implant we placed 8 ISMS microwires in each side of the spinal cord: 4 targeting the EUS inhibitory region and four the bladder preganglionic neurons. The microwire arrays were similar to those shown in our previous report. Before

positioning the array we performed a series of penetrations with a search electrode (30 µm stainless steel microwire) to produce a stimulus/response map of the sacral spinal cord. The responses of interest were changes in bladder pressure, EUS EMG and contractions of intrinsic toe muscles and hamstrings muscles biceps femoris posterior and semitendenosus (PBSt). When the EUS-inhibitory and bladder-excitatory regions had been located, an 8/0 ophthalmic suture was sewn into the dura mater to act as a marker for the rostrocaudal middle of the array.

ISMS Protocol and Data Acquisition

Once the regions of the target nuclei were determined, arrays of 16 stainless-steel microwires were implanted and fixed in place using droplets of cyanoacrylate. A custom-made multi-channel microstimulator was used to deliver ISMS through each electrode in turn (Prochazka et al. 2002). Typically microwires targeting the EUS inhibitory region were inserted 2.5 mm from the dural surface (~1.5 mm from the cord dorsum surface) and those targeting the bladder preganglionic neurons 3.5 mm from the dural surface (~2.5 mm from cord dorsum surface).

RESULTS

1. Correlation between intra-urethral EMG and urethral pressure.

In an acute experiment systematic measurements were made of intra-urethral EMG and pressure for a series of locations of the catheter tip and a series of bladder volumes using the catheter shown in Fig. 4. At each location the bladder was first emptied, then infused with 10 ml increments of saline up to 100 ml. The following intra-urethral locations, expressed in mm distal to the bladder neck, were tested: 0, 10, 20, 30, 40, 50. In each case we switched from bladder to urethral pressure measurement using a two-way valve as shown in the exemplar trace of Fig. 7A.

Fig. 7B shows mean EMGs computed between the dashed vertical lines in Fig. 7A in all of the trials plotted against the corresponding mean urethral pressures. There was no obvious correlation. This confirms the tentative conclusion in our previous report that intra-urethral EMG cannot be used as an indirect measure of intra-urethral pressure. We also confirmed that EMGs recorded simultaneously at different points along the urethra generally differ.

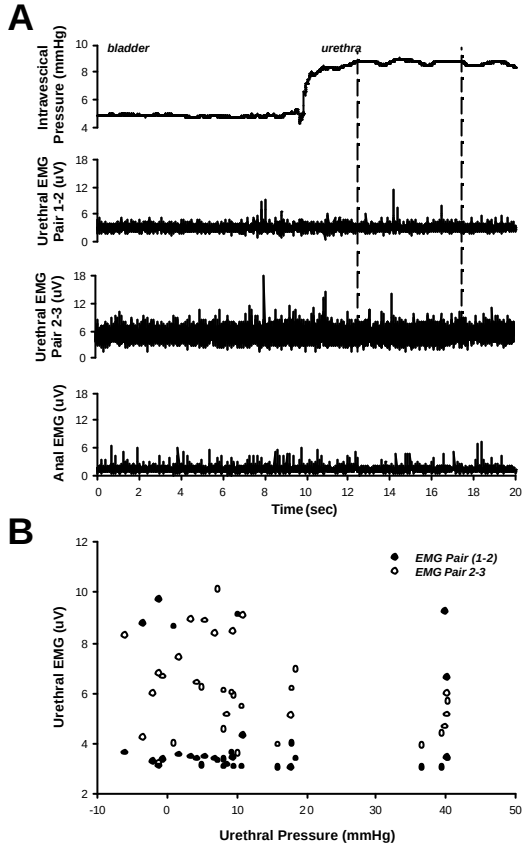

Figure 7 A) Bladder and urethral pressures, urethral EMG between electrodes 1-2 and 2-3, and anal sphincter EMG . B) Average EMG between dashed lines plotted against corresponding urethral pressure for all trials.

2) Bladder and urethral pressure changes evoked by intra-urethral stimulation

We previously noted that intra-urethral stimulation could either stop voiding or, more rarely, evoke it. In the first of our acute experiments in the current quarter we further explored this effect, this time with direct intra-urethral EMG and pressure measurements.

Fig. 8A shows urethral pressure responses to 5 sequential 5s trains of intra-urethral stimulus pulses (4mA, 50/s), applied between Cooner wires #1 and #3 (Fig. 4B). The catheter tip was 17.5 mm from the bladder neck, which meant that the electrodes were centered at ~30 mm from the bladder neck (55 mm from the tip of the penis). Mean urethral pressure consistently increased by about 5-6 mm Hg, though the baseline gradually declined. Fig. 8A shows a similar set of measurements at a slightly lower stimulus strength. The decline in baseline urethral pressure dominated the overall response, pressure dropping nearly 20 mm from the pre-stimulus level. Response B tended to be evoked more readily at higher bladder volumes.

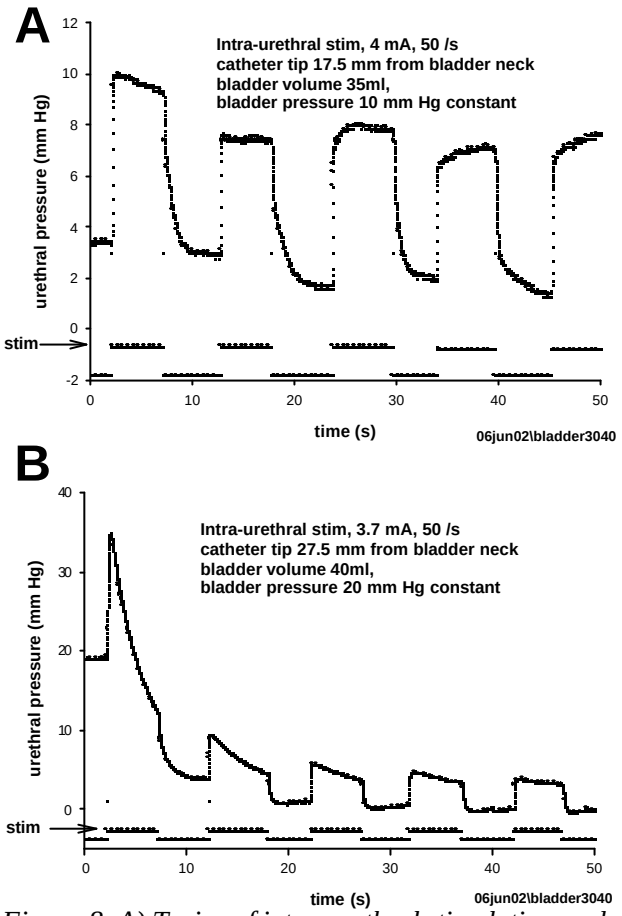

Figure 8 A) Trains of intra-urethral stimulation evoked increases in intra-urethral pressure. B) Stimulation at a lower amplitude evoked transient increases but a large overall decline in urethral pressure.

3) Bladder and urethral pressure changes evoked by intra-urethral stimulation

In the second acute experiment we implanted an array of ISMS microwires as shown schematically in Fig. 9. Electrode positions were referenced to the suture marking the middle of the array (see Methods). The 8 electrodes rostral to the marker (#L1-4, R1-4) were placed within 0.5 mm of the midline to a depth of ~2 – 2.5 mm from the dural surface to target the urethral inhibitory region in the dorsal commissural area at the S1-S2 boundary.

In this experiment stimulation through the implanted microwires was performed under isoflurane anesthesia (2%), at which level EUS reflex responses to bladder distension were present but the cat was unresponsive to noxious somatic stimuli. Changes in bladder and urethral pressure and EMG in response to trains of stimuli through each electrode in turn were recorded. The currents required to produce measurable responses were noted. The microwires eliciting the largest changes were then selected and stimulated together (2 sec trains of interleaved pulses) using 80% of the optimal current for each electrode.

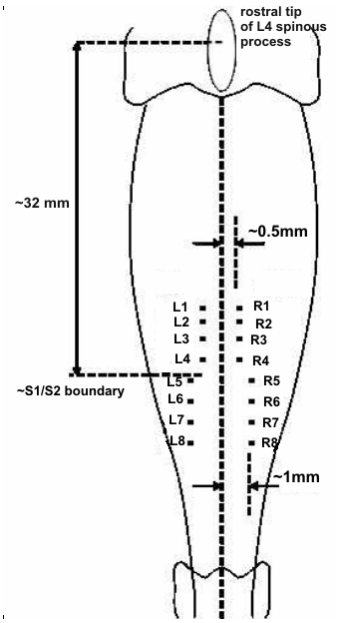

Figure 9 Positions of ISMS microwires in acute experiment 2.

Fig. 10A shows an example of bladder contractions caused by stimulation through electrode L8 in Fig. 9. Unfortunately in this experiment we were unable to evoke consistent inhibition of the urethra after implanting the microwire array, though some inhibition had been observed with the search electrode. 9 microwires elicited bladder contractions in the range 10-25 mm Hg (R4, R5, R6, R7, R8, L4, L6, L7, L8). However these were accompanied by similar increases in urethral pressure as well as increases in urethral EMG (e.g. Fig. 10B). Little or no voiding was evoked in any of the trials.

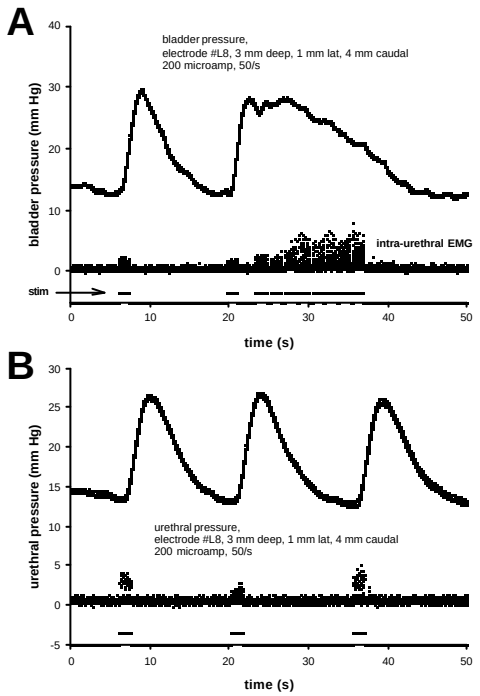

Figure 10. A) Trains of intra-urethral stimulation evoke increases in intra-urethral pressure. B) Stimulation at a lower amplitude evokes transient increases but an overall decline in urethral pressure.

<u>2)Implantation and testing of chronic ISMS microwire array</u>

In the final experiment of the quarter we implanted a cat with an array of 16 ISMS microwires (Fig. 11). As described in the Methods, a modified Foley catheter was implanted in the bladder for pressure measurements (see Fig. 3). A pair of Cooner EMG wires was implanted in the urethra and all leads and tubing were passed subcutaneously to the back-pack where the connectors were stowed (Fig. 2).

Starting the day after the implant, although the cat's overall condition was satisfactory it voided frequently and reacted aversively palpation of its abdomen. Urge incontinence persisted until day 14 when the animal was euthanized with

pentobarbital i.p. Post-mortem dissection did not reveal any obvious infections around the bladder and urethra, but connective tissue had formed solid adhesions around the bladder dome. The urethra seemed intact and infection-free and the intra-urethral EMG wires were in place. There was no evidence that leakage of urine had occurred from the puncture holes around the implants entering the bladder or the urethra. At this point we do not know what caused the urge incontinence. We are considering a fully-implantable telemetric sensor for bladder pressure if this problem continues to arise, because the portion of the Foley catheter that dwells inside the bladder occupies about 5 ml of volume and may irritate the bladder wall. Also, we will terminate the Cooner EMG wires in such a waythat they cannot splay and irritate the interior of the urethra, another possible factor.

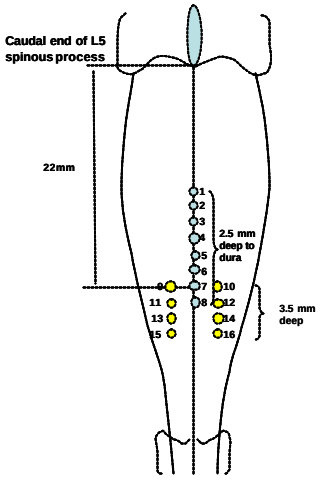

Figure 11 Positions of ISMS microwires in chronic implant, as determined in post-mortem dissection.

Fig. 12 shows an example of a large increase in bladder pressure elicited in the awake cat with ISMS through two of the more caudal electrodes 3 days after implantation of the ISMS microwires. No voiding occurred so the urethral co-contraction was presumably sufficient to retain urine in the face of the large bladder contraction. The cat did not show orienting or aversive responses to stimulation through these electrodes. However it did respond to stimulation through the more proximal electrodes targeting the urethral inhibitory region, so this was discontinued ISMS was tested again 11 days later, but by this time the bladder responses evoked by the electrodes had become much smaller. In this cat we did not succeed in evoking reductions in urethral EMG, either during the implant or after recovery. However we did confirm that large increases in bladder pressure could be evoked in the absence of aversive responses.

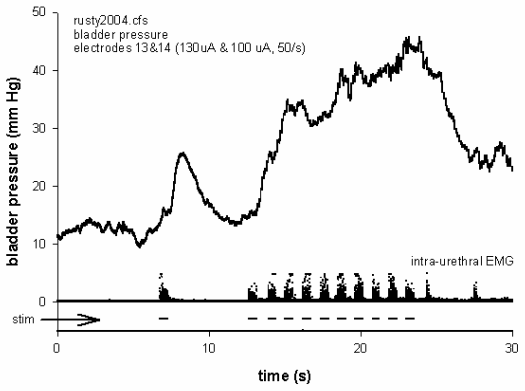

Figure 12 Bladder contractions and intra-urethral EMG responses evoked by simultaneous ISMS (interleaved trains) via electrodes 13 and 14 (Fig. 11) in the awake cat.

PLANS FOR THE NEXT QUARTER

Three chronic ISMS implants in Edmonton

- Characterize the types of bladder and sphincter responses elicited by multichannel ISMS in the sacral region in the awake animal
- Concentrate on eliciting urethral inhibition, either with ISMS or intra-urethral stimulation in combination with bladder contraction to elicit voiding.
- Test a telemetric bladder pressure transducer

The object of these experiments will be to elicit micturition with ISMS in the conscious cat. If this succeeds, these animals will later be transferred to Halifax where they will be spinalized to see whether control over bladder function can be maintained in the chronic spinal state.

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